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# REMOVAL OF OXYTETRACYCLINE ANTIBIOTICS FROM HOSPITAL WASTEWATER

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## ABSTRACT

Harmful effects of antibiotics on the human body are disturbing intestinal balance and gastrointestinal disorders. The aims of this study were to detect the Oxytetracycline (OTC) in the effluent of Wastewater Treatment Plants (WTPs) of Golestan, Abuzar and Taleghani hospitals of Ahvaz, Iran; also, the evaluation of the efficiency of treatment plants for removal of OTC. This study was a cross sectional study. Samples were collected from influent and effluent of the hospitals WTPs in summer, autumn and winter. 42 samples were collected in composite form in all week days. Samples were extracted for Oxytetracycline by passing through SPE column. Ethanol and extracted OTC have been dried with nitrogen gas. Then, OTC inoculated 2 ml methanol and analyzed by HPLC. Highest Oxytetracycline concentration was observed in Taleghani hospital WTP in the summer (0.372 *mg/L*). The maximum removal of percentage was in the summer (97%). Lowest Oxytetracycline concentration was observed in the winter in Abuzar and Golestan hospitals WTPs. Minimum removal percentage of OTC was in autumn for Golestan hospital WTP (10%). Extended Aeration Activated Sludge (EAAS) is capable to remove oxytetracycline, with an average percentage of 53%. Oxytetracycline removal was increased in the hot season and decreased in cold season.

**KEYWORDS:**
Extended Aeration, Activated Sludge, Hospital wastewater, Oxytetracycline.

## INTRODUCTION

In recent years, more pollutants are threaten for the source of drinking water, agriculture and industrial uses in Ahvaz, Iran [1-6]. Antibiotics are organic chemical matters produced by organisms. They kill other organisms, humans, animals and plants [7]. Uptake and excretion of antibiotics are different and depend on antibiotic type [8]. Antibiotics are persistent and lipophilic; they can maintain their chemical structure in body long time for therapeutic purposes [9, 10]. Excessive use of antibiotics may cause bacterial resistance [11]. Since antibiotics have health benefits for humans and animals, indiscriminate use of antibiotics leads to their accumulation in the environment, especially in sewage and hospital wastewater [12-16]. Annually, many antibiotics are consumed worldwide. Europe consumption is about 19.5 packs per 1,000 people [17, 18]. Harmful effects of antibiotics on body include disruptions in intestinal balance and unpredictable consequences on human health and the environment; in fact, it can disrupt the digestive system. Hospital wastewater may contain high levels of pathogenic microorganisms, pharmaceutical residues, metabolites of pharmaceuticals, radioactive elements and other hazardous chemicals [19-21]. Several studies indicate Oxytetracycline presence in hospital wastewater [22-24]. Antibiotics include limited range and wide-ranging antibiotics; Oxytetracycline is broad-spectrum [25, 26]. Oxytetracycline is one of the tetracyclines which is continuously observed in the aquatic environment [27, 25, 28, 29]. Oxytetracycline can be found in surface water, wastewater, groundwater, drinking water, seawater and sediment in all over the world [30, 25, 31, 32, 1, 33]. Presence of oxytetracycline can choose microorganisms variation in natural environments, development of resistance in bacteria or pathogens [30, 25, 34, 35]. Also, Oxytetracycline as growth promoters is used in fish and aquaculture [10, 25]. After a poor absorption, about 70% of Oxytetracycline is excreted from the body through urine and feces [30, 10, 8, 25]. Removal of antibiotics from wastewater include physical processes such as reverse osmosis and nanofiltration systems, chemical processes such as advanced oxidation, and biological processes such as activated sludge system. Biological treatment is cost effective. Among them, activated sludge process is used widely throughout the world [36-40]. Removal of antibiotics through biological treatment is due to bio adsorption [41-43]. Activated sludge process is a biological process of aerobic wastewater treatment which has a good effect on antibiotics removal.

The objective of this study was to evaluate the feasibility of the usage of an extended aeration activated sludge system in removing the Oxytetracycline antibiotics from hospitals wastewater.

## MATERIALS AND METHODS

**Physicochemical parameters.** This research is an experimental study. This study was conducted in the chemical laboratory of the Ahvaz Jundishapur University of Medical Sciences of Iran. In this study, we used to assess the potential effects of PH, Temperature, TSS, Biochemical Oxygen Demand ($BOD_5$) and Chemical Oxygen Demand (COD) on removing the Oxytetracycline antibiotics in Golestan, Abuzar and Taleghani educational hospitals of Ahvaz (located in south-western Iran) [44-47] by using WTPs system during 2016. BOD, COD and TSS values were determined according to the standard methods [48]. Table 1 shows the factors which were affect the influent component including pH, temperature, TSS, $BOD_5$, COD, Qr/Q, MLSS, F/M, SVI, HRT and $Q_c$ characteristics of the wastewater in treatment plant (Table 1). Biosynthetic coefficient commonly for activated sludge process in WWTP showed in table 2.

**TABLE 1**
**The influent component of the wastewater in treatment plant (WWTP) of Golestan, Abuzar and Taleghani educational hospitals**

| parameter | Unit | Wastewater(average) |
|---|---|---|
| **pH** | - | 7.95 |
| **Temperature** | $mgL^{-1}$ | 23.8 |
| **$BOD_5$** | $mgL^{-1}$ | 250 |
| **COD** | $mgL^{-1}$ | 510 |
| **TSS** | $mgL^{-1}$ | 500 |
| **Qr/Q** | - | 64.28 |
| **MLSS** | mg/l | 2819.36 |
| **F/M** | $d^{-1}$ | 0.35 |
| **SVI** | ml/g | 144.89 |
| **HRT** | hr | 6 |
| $\theta c$ | d | 10.93 |

**TABLE 2**
**Biosynthetic coefficient commonly for activated sludge process in WWTP**

| coefficient | Unit | Range |
|---|---|---|
| **K** | grCOD/grVSS.d | 5 |
| **$K_s$** | mg/l BOD | 60 |
| **Y** | mg biomass/mg $BOD_5$ | 0.6 |
| **$K_d$** | grVSS/grVSS.d | 0.1 |
| **$\mu_{max}$** | $d^{-1}$ | 1 |

**Sampling.** Samples were collected from the influent and effluent of the wastewater treatment plants at 3 educational hospitals during 2016. In this study 42 samples were collected with two glass bottles in 1000 ml volume. Then we added Mac Ilvain solution (5 ml) to the samples (dissolve OTC). In the next stage, wastewater was filtered with a filter paper placed in a Buchner funnel to remove suspended solids and impurities from wastewater sample. At the end of the sampling, the SPE was dried with the nitrogen gas. Samples are inoculated with 2 ml methanol and analyzed by HPLC. Wastewater sampling was a 24- hour composite sampling. Sampling was performed in 3 hospitals, nine samples for warm season (July, August and September), six samples for temperate season (November and the first half of November), six samples for cold season (second half of December and January), respectively that had the same biological treatment (Extended aeration activated sludge). Sampling was done every day from Saturday to Friday. In this study for OTC pollutant 42 samples of 3 educational hospitals were taken and collected during 2016.

**Chromatogram from analysis of OTC.** Chromatography is commonly applied to determine low levels of antibiotics in water samples. The identification of drug candidates or metabolites usually involves the application of MS and HPLC systems (Fig.1) [49]. A chromatogram for a mixture of OTC is presented in Figure 1. Peaks of OTC were observed at the retention times of 2.4 min (Figure2) [48].

## RESULTS AND DISCUSSION

**Removal of OTC.** This study was conducted in 3 main educational hospitals affiliated to Ahvaz Jundishapur University of medical sciences, Iran during 2016. Various OTC removal efficiencies were obtained in the various studies. Based on the results, OTC concentrations were generally reduced (70-100%) by the wastewater treatment process at WWTPs [50]. Based on the result of our study, reported percent reductions of OTC were as 67.9-100. But studies have also shown limited, below 30%, to no reduction [51-54].

OTC

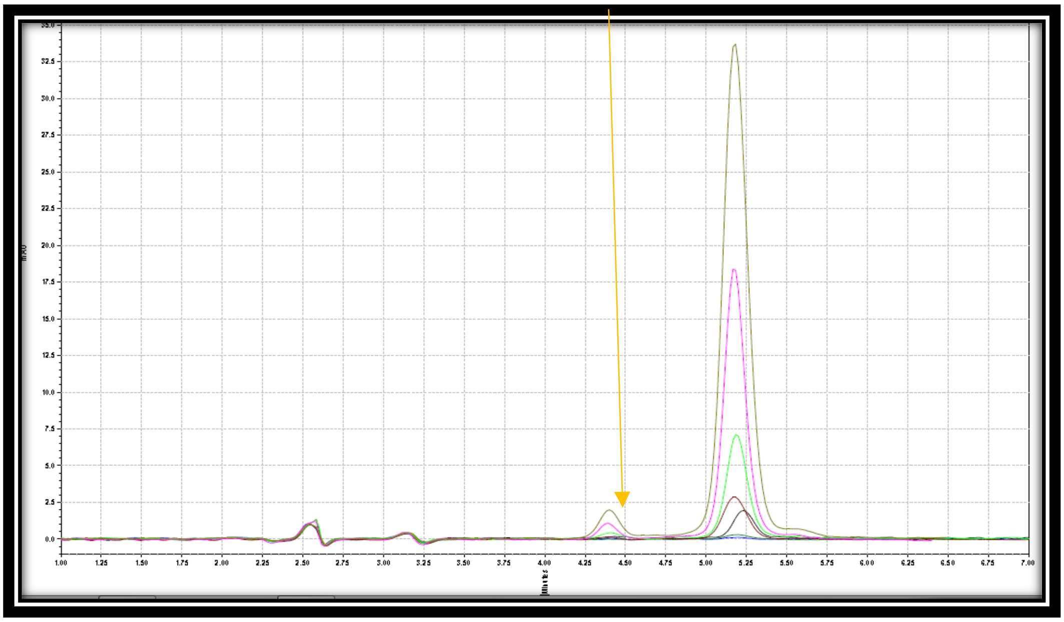

**FIGURE 1**
**Chromatograms of quantitation of ions for standard OTC**

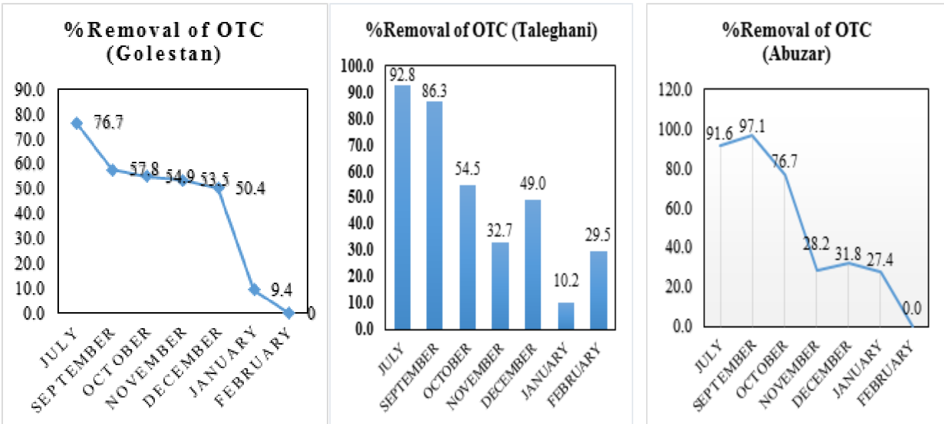


**FIGURE 2**
**OTC removal efficiencies of the WWTP**

Antibiotic removal efficiency is affected by several factors, including (I) specific treatment processes employed by individual sewage treatment plants; (ii) the sewage residence time at different sewage treatment plants; and (iii) chemical structures and properties of the antibiotics [53]. The persistence of antibiotics determines their removal capabilities during treatment processes. The high removal rates of OTC may be attributed to their tendencies to adsorb quickly to soils, sediments and sewage sludge [55, 56, 53]. In the present study the average removal of OTC in Golestan, Taleghani and Abuzar was 50%, while 76% had the highest removal, 50%, while 92% was the highest removal and 58%, while 97% reported of the highest removal in the hospital, respectively (Fig. 2). The highest removal rate is Abuzar hospital. As well as the Abuzar hospital has the highest average removal of OTC. It should be noted that the top removal of OTC antibiotics in biological treatment processes for wastewater temperature is high. Higher temperature increases the activity of microorganisms in wastewater. Also, due to more OTC, the activity of microorganism is eliminated.

**Removal of physicochemical parameters.** The maximum pH value of the influent of WWTP was 9.1. Generally, pH values of the influent were between 8.0 and 9.0. The maximum pH value of the effluent of WWTP was 7.2. The pH value of the effluent was between 7.0 and 8.0. The PH values of the influent were higher than the pH values of the effluent. The maximum temperature value of the influent was 29$^{O}$C. The activated sludge processes

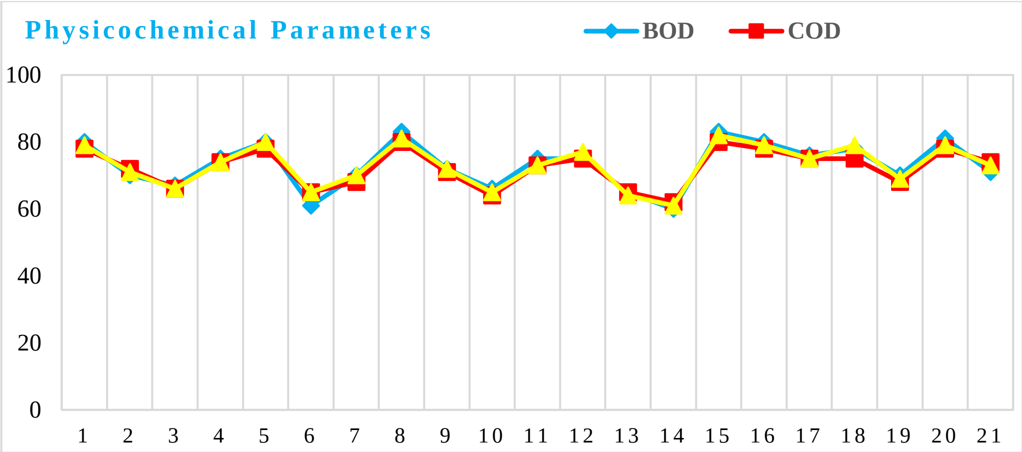


**FIGURE 3**
**Physicochemical parameters determined in the WWTP in Golestan, Abuzar and Taleghani educational hospitals**

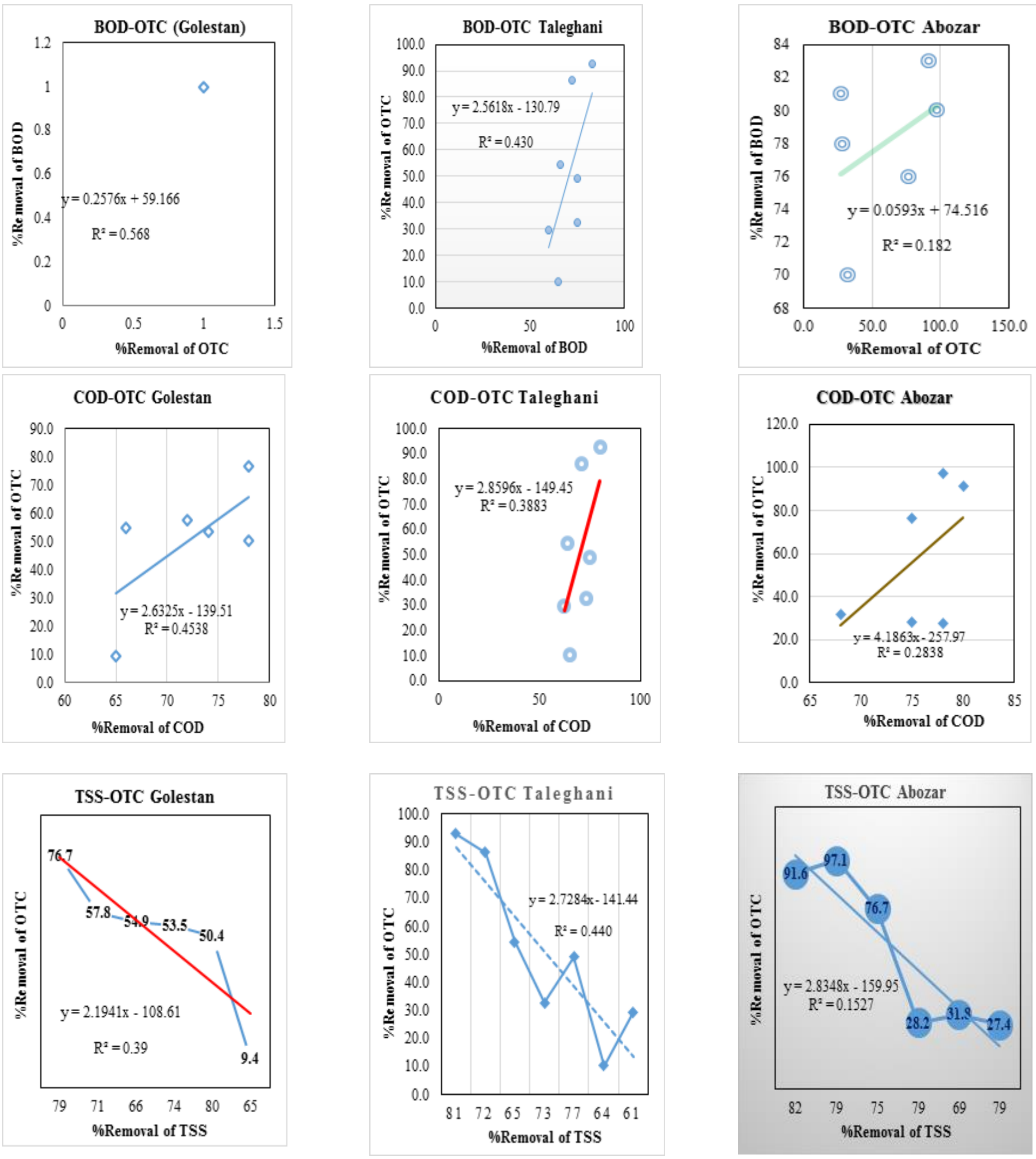


**FIGURE 4**
**Relationships between removal efficiencies of physicochemical and biological parameters (TSS, BOD5, COD and OTC)**

are widely used in biological treatment of municipal and industrial wastewater for its highly efficient removal of organic matter, nitrogen and phosphorus; that is the most commonly used process to treat wastewater worldwide [57, 48, 58].Maximum removal efficiency of $BOD_5$ was obtained as 80% in Golestan hospital. The $BOD_5$ removal efficiency of the treatment plant was over 71%. Maximum removal efficiency of $BOD_5$ was obtained as 83% in Taleghani hospital. The $BOD_5$ removal efficiency of the treatment plant was over 70%. Maximum removal efficiency of $BOD_5$ was obtained as 83% in Abuzar hospital. The $BOD_5$ removal efficiency of the treatment plant was over 77% (Figure 3). Maximum removal efficiency of COD was obtained as 78% in Golestan hospital. The COD removal efficiency of the treatment plant was over 71%. Maximum removal efficiency of COD was obtained as 80% in Taleghani hospital. The COD removal efficiency of the treatment plant was over 70%. Maximum removal efficiency of COD was obtained as 80% in Golestan hospital. The COD removal efficiency of the treatment plant was over 75 % (Figure 3). The high removal rates for COD and $BOD_5$ are caused by sedimentation of TSS [59]. Maximum removal efficiency of TSS was obtained as 80% in Golestan hospital. The average TSS removal efficiency of the treatment plant was over 72%. Maximum removal efficiency of TSS was obtained as 81% in Golestan hospital. The average TSS removal efficiency of the treatment plant was over 70%. Maximum removal efficiency of TSS was obtained as 82% in Golestan hospital. The average TSS removal efficiency of the treatment plant was over 76 % (Figure 3).

**Relationships between removals of OTC and physicochemical parameters.** Relationships between removal efficiencies of physicochemical parameters ($BOD_5$, COD, and TSS) and OTC were determined. In terms of the relationships between removals of TSS and OTC, correlation coefficient ($R^2$ = 0.39) was obtained for OTC in Golestan hospital and the correlation coefficient ($R^2$ = 0.44) was obtained for OTC in Taleghani hospital. The correlation coefficient ($R^2$ = 0.15) was obtained for OTC in Abuzar hospital. The order of importance of relationships between removal efficiencies of TSS and OTC (Golestan), OTC (Abuzar), OTC (Taleghani) was $OTC_T > OTC_G > OTC_A$ (Figure 4). In terms of the relationships between removals of COD and OTC, correlation coefficient ($R^2$ = 0.45) was obtained for OTC in Golestan hospital. The correlation coefficient ($R^2$ = 0.38) was obtained for OTC in Taleghani hospital. The correlation coefficient ($R^2$ = 0.28) was obtained for OTC in Abuzar hospital. The order of importance of relationships between removal efficiencies of COD and OTC (Golestan), OTC (Abuzar), OTC (Taleghani) was $OTC_G > OTC_T > OTC_A$ (Figure 4). In terms of the relationships between removals of BOD and OTC, correlation coefficient ($R^2$ = 0.56) was obtained for OTC in Golestan hospital. The correlation coefficient ($R^2$ = 0.43) was obtained for OTC in Taleghani hospital. The correlation coefficient ($R^2$ = 0.18) was obtained for OTC in Abuzar hospital. The order of importance of relationships between removal efficiencies of BOD and OTC (Golestan), OTC (Abuzar), OTC (Taleghani) was $OTC_G > OTC_T > OTC_A$ (Figure 4). The removals of physicochemical parameters were weakly correlated with OTC [50].

## CONCLUSION

In this study, a detailed analyzed data were carried out to find the removal of Oxytetracycline at Golestan, Abuzar and Taleghani hospitals educational hospitals of Ahvaz (located in the south-west of Iran), during 2016. The OTC Consumer is low in three hospitals of Golestan, Taleghani and Abuzar. The OTC has changed from warm and temperate to cold weather increases the OTC in addition to infectious diseases, burns and surgical applications. Wastewater treatment with extended aeration can good remove of OTC (53%). Range of OTC removal is 9% to 96% with extended aeration. The lowest range of OTC during the week was on Friday and Saturday and Sunday had the high OTC. High OTC in extended aeration was associated with WWTP equipment. Controlling the OTC influent, application of modern equipment and careful monitoring will have important roles in increasing removal of OTC.

## CONFLICT OF INTERESTS

Authors have no conflict of interests.

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